

1 **Spatial scale determines how the morphological diversity relates with river**  
2 **biological diversity. Evidence from a mountain river in the central Chilean Andes.**

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12 macroinvertebrates, fish, anurans, Chile.

13  
14 **ABSTRACT**

15 Rivers shape the landscape and determine spatial connectivity for a variety of riverine  
16 and terrestrial organisms. Rivers impacted by human disturbances are often in need of  
17 restoration in order to increase their functionality and ecological diversity, and  
18 ultimately to improve the ecosystems services that they offer. Because it is usually  
19 assumed that physical diversity of river systems promotes biological diversity or riverine  
20 species, river restoration practices often tend to enhance simplified rivers by increasing  
21 structural morphological complexity of river reaches. However, the relationship between  
22 the variability of physical features and the biological communities in riverine  
23 environments is not necessarily straightforward to assume due to the high degree of  
24 complexity and feedback in the relationship. This work presents a case study with the  
25 application of a recent geomorphological method developed in Europe for the  
26 assessment and characterization of geomorphic conditions in combination with  
27 biological surveys, along a mountain river basin in Central Chile (Clarillo River).  
28 Although representing a single “snapshot” in the pulsating nature of a river system, our  
29 results suggest that availability of habitats provides the conditions to support different  
30 levels of biodiversity in a hierarchical way in terms of spatial scale. In particular, we  
31 found that abundance and diversity of macroinvertebrates are more related with

composition of geomorphic units, whereas the presence of fish is more related with the geomorphic nature of the reaches, and the presence of anurans is more related with the geomorphic nature of river segments.

## **1. Introduction**

Rivers shape and determine the spatial connectivity of the landscape, providing a variety of resources and services (e.g. Brierley *et al.*, 2013). However, rivers are often modified and impacted to the point that the environmental services they provide are severely affected (Wohl, 2014), and in need of restoration (Wohl *et al.*, 2015). River restoration practices often tend to enhance simplified and ecologically poorly functional rivers by increasing structural morphological complexity of river reaches, with the aim to increase the potential availability of habitats and hence the presence of target organisms or communities (Wohl *et al.*, 2015). The underlying ecological assumption is that physical environmental diversity would promote biological diversity (e.g., Frissell *et al.*, 1986; Thorp *et al.*, 2006). Although this assumption is reasonable and often demonstrated, several studies revealed that this is not always the case (e.g. Sullivan *et al.*, 2004; Palmer *et al.*, 2010; Eskew *et al.*, 2012; Milner *et al.*, 2015; Schumtz *et al.*, 2016; Gowns *et al.*, 2017). Indeed, river biodiversity depends on a variety of drivers at small and large scales, such as fluvial processes, physico-chemical features, biological conditions (e.g., competition and predation) and land use (e.g. Friberg *et al.* 2009; Leps *et al.*, 2015). Our knowledge on the ecological interactions between hydrological, geomorphological and biological processes is increasing and informing river restoration practices (Palmer & Ruhi, 2019). However, the relationships between physical and biological features in rivers remains poorly understood, mainly because surveys for physical habitat are not likely to coincide in a spatio-temporal scale to biological sampling (Rasmussen *et al.*, 2011). A deep understanding of ecomorphological relationships between morphological and biological diversities in rivers is yet to be attained.

The morphological characteristics of a river system can be assessed using several protocols, and it is commonly quantified in order to support watershed managers in taking strategic decisions about river management and restoration priorities (Fryirs & Brierley, 2013; Rinaldi *et al.*, 2015; Wheaton *et al.*, 2015; Gurnell *et al.*, 2016). Fluvial

63 geomorphological studies usually aim at understanding the river dynamics and processes  
64 at different spatio-temporal scales (Brierly & Fryirs, 2005; Rinaldi *et al.*, 2015; 2017). In  
65 Europe, especially after the implementation of the EU Water Framework Directive  
66 2000/60/EC, a variety of assessments, protocols and methodologies for river  
67 hydromorphological assessment have been developed (Belletti *et al.*, 2015).

68 From the fluvial ecology perspective, the distribution of species occurs as a longitudinal  
69 transition depending on stream dimensions and type (Vannote *et al.*, 1980), the regime  
70 of flow and flood pulse (Junk *et al.*, 1989; Tockner *et al.*, 2000), and the presence of  
71 single habitats. Furthermore, temporal and spatial scales of connectivity are currently  
72 considered as major drivers in the development of ecological functions in rivers (Poepl  
73 *et al.*, 2020). Ecological conditions and diversity in river systems depends on the degree  
74 of relations between the biotics and abiotic components, and within biotic communities,  
75 which are often assessed based on benthic macroinvertebrates (Thomson *et al.*, 2004),  
76 although other groups such as diatoms, aquatic macrophytes, fish and terrestrial  
77 invertebrates should be taken into account as indicators for the overall river ecological  
78 and environmental conditions (Friberg *et al.*, 2009; Golfieri *et al.*, 2015; Kärnä *et al.*,  
79 2019).

80 The relationship between the variability of physical features and the biological  
81 communities in riverine environments have long been investigated (Frissell *et al.*, 1986;  
82 Maddock, 1999), and it has been recognized that fluvial geomorphology is one of the  
83 main drivers for the fluvial biota (Kaller & Hartman, 2004; Poole *et al.*, 2010). Attempts  
84 to test explicitly whether biotic communities are consistently different between different  
85 geomorphic units or different river styles suggest that geomorphic characteristics must  
86 be considered within the larger scale variables and processes that determine the presence  
87 and dynamics of such characteristics (e.g. Thomson *et al.*, 2004; Chessman *et al.*, 2006).  
88 Combining morphological indexes used in streams characterization systems with biotic  
89 indexes remains indeed a key issue for researchers, as the spatio-temporal differences in  
90 riverine communities will also depend on other variables, such as climate, water quality,  
91 and hydrological regimes amongst others (Golfieri *et al.*, 2015; Palmer & Ruhi, 2019).  
92 This makes difficult to verify and quantify the role of physical drivers on river  
93 ecosystem conditions and implies that field sampling and classification procedures

94 should be both biologically and geomorphologically meaningful (Brierley *et al.*, 2013).  
95 Advances in this sense have been made since the River Styles Framework (RSF,  
96 Brierley & Fryirs, 2005; Fryirs & Brierley, 2013), which surveys at the basin scale the  
97 physical setting and morphology of rivers (i.e., the river character), the magnitude and  
98 dynamic of changes (i.e., the river behavior) and assess future evolutionary trajectories  
99 (see also the multi-scale hierarchical framework developed in Europe by Gurnell *et al.*,  
100 2016). Similarly, the Morphological Quality Index method (MQI; Rinaldi *et al.*, 2015)  
101 and the Geomorphic Unit survey and classification System (GUS; Belletti *et al.*, 2017)  
102 attempt at integrating information on the hydrology, fluvial geomorphology, riparian  
103 vegetation, and human pressures at a variety of scales, and provide a  
104 geomorphologically-sound template to study geomorphology-biology relationships.  
105 This work presents a case study of the application of a geomorphological survey method  
106 for the assessment and characterization of geomorphic conditions (Rinaldi *et al.*, 2017)  
107 in combination with biological surveys, along a mountain river basin in Central Chile  
108 (Clarillo River). The aim is to explore the relationships between geomorphic and  
109 biological diversity in a relatively low-impacted river, in order to contribute towards the  
110 understanding on how river communities such as amphibians, fish, and  
111 macroinvertebrates are influenced and distributed into the geomorphic complexity of the  
112 fluvial ecosystem, at different spatial scales. Specifically, we intend (i) to quantify  
113 differences in the communities between different spatial scales, i.e. segments, reaches,  
114 geomorphic (or geomorphological) units and microhabitats, characterized by  
115 homogeneous geomorphic conditions in a river with negligible human impacts; and (ii)  
116 to provide evidences of the link between biological and geomorphological diversity. The  
117 available knowledge on relationship between morphological and biological diversity in  
118 rivers is usually gathered in studies conducted in developed countries, with very scarce  
119 evidence available for rivers in developing countries. According to Andreoli *et al.*  
120 (2012), Chilean rivers show a high hydromorphological variability, and are poorly  
121 understood and generally ill-managed. Central Chile is an area of national concern, since  
122 it is where the water related issues re-emerged due to population growth and this is an  
123 hotspot area for biological conservation. In this sense, the case is paradigmatic for  
124 several countries with a fast-growing economy.

125

## 126 **2. Methods**

127 The study was carried out in the Clarillo basin, located in the central Chilean Andes  
128 (Figure 1). The Clarillo River basin drains an area of 367 km<sup>2</sup>, and ranges in elevation  
129 from 609 to 3057 m a.s.l. The climate is typically Mediterranean, and the mean annual  
130 rainfall is around 300 mm. Approximately 63 percent of the basin is covered by  
131 xerophilic woods (below 1600 m.a.s.l) and shrub lands (Alarcon et al., 2015). The upper  
132 part of the basin (131 km<sup>2</sup>) is protected since 1982 as Reserve, managed by the Chilean  
133 National Forest Corporation (CONAF). The present study mainly focused on the main  
134 Clarillo River near the closing section of the Reserve (Figure 1), where the river is  
135 mostly alluvial, although confined by hillslopes and ancient terraces. The river features  
136 morphologies typical of high-gradient streams such as step-pool, cascade, and riffle-  
137 pool. In particular, the analysis focused on three segments (A, B, and C towards  
138 downstream direction), located near the entrance of the Reserve for a total length of  
139 approximately 9 km. On each segment, three reaches were selected (Figure 2), and all  
140 field surveys were conducted at this scale between September 2016 and May 2017,  
141 resulting in nine survey sites (reaches A1 to C3). The reaches were comparable in terms  
142 of length (on average 190 m), width (on average 9.4 m), and slope (on average 7%) and  
143 their main difference is the elevation (ranging from 814 to 1139 m a.s.l.).

### 144 *2.1. Morphological surveys*

145 In order to gain a general geomorphological understanding of the river system, the  
146 Morphological Quality Index (MQI; Rinaldi *et al.*, 2015) was initially applied to the  
147 whole river network of the Clarillo basin. The MQI is a method to evaluate  
148 morphological conditions through a multiscale hierarchical framework based on a series  
149 of 28 indicators related to features such as functionality, artificiality, and recent channel  
150 adjustments. The MQI was applied at the basic spatial unit for the evaluation of  
151 morphological conditions which corresponds to a section of a few kilometers in length  
152 (named segment in this paper). We used this step to identify, through a  
153 geomorphologically-based procedure, the three segments on which perform the analyses  
154 described below. The method combines data from remote sensing (satellite images from  
155 Google Earth in this study), GIS analysis and field surveys.

156 At a smaller scale, three reaches were selected per each of the three segments (Figure 1  
157 and 2; Table 1). At the scale of reach we applied the Geomorphic Units survey and  
158 classification System (GUS; Belletti *et al.*, 2017) to characterize the geomorphological  
159 nature of the sites. The basic spatial unit at which the method classifies distinctive  
160 morphological characteristics is the geomorphic unit, which corresponds to the  
161 mesohabitat scale (i.e. about  $10^0$ - $10^2$  m). Within a geomorphic unit, the sub-units  
162 correspond to the microhabitat scale, i.e. a characteristic patch within a unit (about  $10^{-1}$ -  
163  $10^1$  m; Figure 2; Belletti *et al.*, 2017).

164 In the Clarillo River, the GUS was applied at the scale of geomorphic units (Basic level;  
165 Belletti *et al.*, 2017). The survey of geomorphic units mainly focused on the bankfull  
166 channel, given the absence of a continuous and extensive floodplain as the river is  
167 confined in its valley. The location, type, and number of each geomorphic unit was  
168 recorded in the field for each reach. Their identification was supported by remote-  
169 sensing analysis on the basis of high resolution vertical close-range photos obtained in  
170 the field using a Phantom 3 drone (DJI Technology Co., Ltd, USA) equipped with a  
171 FC300X camera (resolution of 4000x3000 and pixel size of 1.56x1.56  $\mu$ m, for a ground  
172 resolution of 7.55 mm/pixel). The flying altitude ranged between 12.6 and 63 m. A  
173 detailed orthophoto and elevation digital model of each reach were created using  
174 Structure-from-Motion (SfM) technique, using the Agisoft PhotoScan® professional  
175 software. The geomorphic units were then classified on the orthophotos by photo-  
176 interpretation and measured. Then we calculated the diversity and abundance of  
177 geomorphic units at three scales (segment, reach and geomorphic unit), adapting GUS  
178 metrics and indexes from Belletti *et al.* (2017). These indexes illustrate the presence,  
179 number, and spatial variability of the main geomorphic units found in the study site  
180 (cascade, pools, steps and riffles; Figure 3) at those different scales. We calculated the  
181 following:

- 182 - the abundance (N), proportion (%) and areal proportion (% area) of each  
183 submerged unit type (cascades, pools, steps and riffles);
- 184 - the richness of geomorphic units (GUs), as sum of types of GUs ( $\sum NT_{GU}$ );
- 185 - the number of GUs (any type;  $\sum N_{GU}$ );
- 186 - a modified Geomorphic Unit Richness within the bankfull channel (mGUSI-

- 187  $R_{BC}$ ), as the sum of types of geomorphic units (GU) observed in a reach in  
 188 comparison to the maximum number of possible unit types (submerged and  
 189 emerged units within the bankfull;  $N=24$ );
- 190 - the Geomorphic Unit Density (GUSI-D) index, that is the sum of GUs (any type;  
 191 in that case only for bankfull channel units) divided by the reach length;
  - 192 - a modified Geomorphic Unit Density (mGUSI-D\_A) index where the sum of  
 193 GUs (any type) is rated by reach area.

194 We also calculated sub-indexes differentiating between submerged and emerged units:

- 195 - a richness index of submerged units, the GUSI- $R_C$ , calculated as sum of types of  
 196 submerged GUs observed in comparison to the maximum number of possible  
 197 types of submerged units;
- 198 - the index of density of submerged units, the GUSI- $D_C$ , calculated as number of  
 199 submerged GUs divided by the area of the submerged units;
- 200 - a modified sub-index of density of submerged units, the mGUSI- $D_C$ , where the  
 201 number of submerged GUs is rated by the reach length;
- 202 - the sub-index of density of emerged units, the GUSI- $D_E$ , calculated as number of  
 203 emerged GUs in comparison to area of these units.

## 204 *2.2. Biological surveys and microhabitat scale characterization*

205 Benthic macroinvertebrates, fish, and amphibians were surveyed at the scale of reach. A  
 206 Surber sampler (mesh 500 $\mu$ m; Figure 4) was used for the benthic macroinvertebrates  
 207 surveys. The sampler covered an area of 0.09 m<sup>2</sup> (per sample) and was operated by  
 208 manually rinsing sediments for five minutes within the sampling area before collecting  
 209 the sample of macroinvertebrates. Eight samples were collected on each reach, at points  
 210 randomly distributed across the different types of in stream geomorphic units (pools,  
 211 riffles, and cascades), thus resulting in a total of 24 samples over 2.16 m<sup>2</sup> per segment  
 212 and 72 samples overall. Macroinvertebrate samples were conserved in alcohol 70% and  
 213 taken into laboratory for identification to the taxonomic level of family, though a small  
 214 part of the specimens were identified to the level of species.

215 The ichthyofauna within each reach was surveyed using the standard electrofishing  
 216 method, using a backpacker Electrofishing Hatech Equipament (Haltech Aquatic  
 217 Research Inc. Ontario, Canada; Figure 4). Two passes of approximately 40 min or one

pass of 60 min were conducted in each of the reaches, in the direction downstream-to-upstream. The collected fish were identified in the field to the level of species, and then measured and weighted, before releasing them back alive in the same sampling reach (Figure 4).

As to the amphibians, we used the Visual Encounter Survey and Time Constrained Search method. A systematic nocturnal survey of one hour was conducted in two transects covering the entire reach section, searching in potential habitats for the local species. When specimens were found, they were identified to the level of species, counted and released in the same reach in their respective potential refuge. The present investigation focused exclusively on adult individuals (Figure 4).

At the microhabitat scale, microhabitats conditions such as water depth (m) and flow velocity ( $\text{m}^3/\text{s}$ ) were measured at the points of macroinvertebrate sampling using a tape and a propeller, respectively. At both the microhabitat scale and across the reaches, the grain size distribution was obtained using the traditional grid-by-number procedure at submerged and emerged units. Additionally, a multiparameter YSI (YSI Inc. Ohio, USA) sonde was used to measure conductivity, dissolved oxygen, pH, temperature, total dissolved solids on a random point at each reach.

### 2.3. Data analysis

The metrics used for the quantitative measurement of biodiversity were the richness (number of taxa; S) and abundance (number of specimens per each taxa, N). We also calculate the Shannon-wiener Index (H) which is accounting for the proportion of taxa abundances in comparison to the total number of taxa (calculated using natural logarithm). We also calculated the Simpson Index (1-D) as the proportion of taxa abundances relative to the total number of taxa and squared, and this indicates diversity through a relative dominance for each species. Furthermore, we calculated the Evenness (J) and the Margaleff index (D), which indicate the proportion of richness in comparison to abundance. Furthermore, for the macroinvertebrates we calculated the proportion of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT, broadly used as bioindicators), and the abundance of specimens from the family Chironomidae (a particularity tolerant family of macroinvertebrates). Also, an index of relative preference of habitats was adapted from Sullivan *et al.* (2004), which was calculated using the



number of individuals per taxa found in each type of unit in comparison to the total number of individuals. This was calculated exclusively for macroinvertebrates from each of the families identified, in order to associate directly the benthic macroinvertebrate community to geomorphic units. For fish, we calculated the capture per unit effort (CPUE), considering sampling length and time of field survey associated to abundance of specimens captured, their size and weight, which were considered as biological traits of life cycle.

We compared the biological indexes at the segment, reach and geomorphic unit (GU) scale by adopting an ANOVA test to find significant differences among the samples. To identify the relationships between the geomorphological (i.e. the indexes and sub-indexes of the GUS protocol) and the biological variables at three different scales (segment to GU), we used Pearson's correlation. Further, multiple lineal regression analyses were used to quantify significant relationships. Spearman's  $r$  correlations were used for nonparametric data to explore for potential nonlinear relationships. All data was tested at the  $\alpha = 0.05$  level. However, a cut value (i.e.  $r > 0.5$ ) was used to verify correlation. All statistical analysis was performed using Statistica 7 software.

### **3. Results**

#### *3.1. Morphological Quality Index survey (MQI) at the scale of segments*

The whole river network of the Clarillo basin was divided into 21 homogeneous segments. The MQI was assessed at the segment scale, and the values ranged from poor (0.47) to very good or high (0.97) (Table 2). Morphological values were higher in the upper part of the basin (located within the Reserve; segments 9 to 18) than in the lower part where there are water extraction points and other human activities and infrastructures within the riverine area (segments 1 to 3). The three selected segments investigated in this study (A, B, and C) are located within the natural Reserve and are mainly single-thread, straight, confined channels (Table 2). These segments were classified as very good ( $0.8 < \text{MQI} < 1$ ) but did not reach the highest value because they lack potential sources of heterogeneity according to MQI characterization standards (e.g. large wood), and because of the presence of minor infrastructures (e.g. a small wooden bridge crossing one of the segments). The slope of these segments ranges from 0.02 to

0.17 m m<sup>-1</sup> and their length from 2.8 to 3.8 km. The selected segments feature mainly geomorphic units as cascades, riffles, steps, pools and bank-attached bars. Locally, small vegetated islands, mid-channel bars, and narrow floodplain pockets were also present.

### 3.2. Geomorphic Unit Survey (GUS) at the scale of reaches and segments

Figure 5 shows an example of the application of the GUS survey to reach C2. The reaches ranged from 130 to 230 m in length, from 7.8 to 11.12 m in width, and from 814 to 1139 m a.s.l. in terms of elevation (Table 1). Sediments sizes range from sand to boulders, although the dominant median grain size ( $D_{50}$ ) was coarse gravel (Table 1).

Overall, 242 submerged geomorphic units and 284 emerged units were identified within the bankfull channel across the nine surveyed reaches. The portion of reaches covered by submerged morphological units ranged between 67 to 91% of the bankfull area. The most abundant type of submerged unit was pool, followed by step, representing together the 76% of all submerged units (Table 3). The emerged unit types with the highest and lowest abundance were bank-attached high bar and small vegetated island, with 53 and 14%, respectively.

Steps were the most extended submerged morphological feature on reach A1 (38% of submerged units), followed by cascades (17%), riffles (12%) and pools (11%). In reach A2 and A3 the most extended submerged units were cascade and steps, accounting for 49 and 30% of the submerged areas, respectively (Table 3). In B1, the morphological unit cascade represented 47% of the submerged portion of the reach. Cascade was the most extensive morphological unit in B2 and B3 as well, with percentages of 53 and 47, respectively. In the reaches composing the segment C, cascade was again the morphological unit occupying most of the submerged portion of the river, accounting for 51, 47 and 42% of reaches C1, C2, and C3, respectively (Table 3).

As to the GUS indexes at the reach scale (Table 3), the reach C3 featured the highest richness values ( $\sum NT_{GU}=10$ ;  $GUSI-R=0.42$ ), while reach C2 had the highest abundance of geomorphic units ( $\sum N_{GU}=82$ ). As expected, richness indexes are quite homogeneous across the study area (Table 3). Linear density values are highest in reach B1 and lowest in reach B2 ( $GUSI-D=376.92$  and  $191.3$  n/km, respectively). Areal density values are homogeneous across the study area, with lowest values for submerged and emerged units in reach C3 and C1, respectively ( $GUSI-D_C=0.01$  n/km<sup>2</sup>;  $GUSI-D_E=0.021$  n/km<sup>2</sup>).

311 Considering the GUS metrics averaged at the scale of entire segments, in general  
312 richness and density of geomorphic units slightly increased from A to C. The most  
313 important increment towards the downstream direction is recorded for overall linear  
314 density, where GUSI-D ranged from 304.5 to 341.22 n/km, and GUSI-D<sub>E</sub> ranged from  
315 0.06 to 0.21 n/km<sup>2</sup>.

316 *3.3. Benthic community at the scales of microhabitat, geomorphic units, reach and*  
317 *segment.*

318 A total of 5274 specimens of macroinvertebrates were sampled in the field surveys.  
319 Representatives of 11 orders and 30 families were identified. At the order level, the  
320 dominant taxa were Dipterans (33%), Ephemeropterans (33%) and Trichopterans (22%),  
321 followed by Coleopterans (7%). At the family level, the dominant taxa were  
322 Leptophlaebidae, Baetidae, Chironomidae, Hydropsichidae, and Athericidae.

323 At the microhabitat scale, there was a positive and significant relationship between water  
324 depth and macroinvertebrates richness ( $r=0.67$ ;  $r^2=0.45$ ;  $p<0.05$ ). Flow velocity was  
325 inversely and significantly related with several diversity metrics including the Shannon-  
326 Wiener ( $r=-0.76$ ,  $r^2=0.59$ ,  $p<0.05$ ), the Simpson ( $r=-0.8$ ,  $r^2=0.64$ ,  $p<0.05$ ) and the  
327 Evenness indexes ( $r=-0.9$ ,  $r^2=0.82$ ,  $p<0.05$ ), showing that less families are able to  
328 colonize and live in morphological units characterized by higher flow velocity.

329 At the scale of geomorphic units, none of the biological metrics showed statistically  
330 significant differences. For example, Figure 6 shows that the abundance and richness of  
331 macroinvertebrates is not significantly related with the type of submerged morphological  
332 unit ( $p>0.05$ ). The relative preference index (see Sullivan *et al.*, 2004) indicates that  
333 Hydrobiidae, Athericidae, Glossosomatidae, Ceratopogonidae, Elmidae and  
334 Hydroptilidae are more easily associated with pools, whereas Lumbriculidae,  
335 Chironomidae, Leptophlaebidae and Hydropsichidae are more likely found in riffles,  
336 and Gripopterygidae, Gomphidae, Tipulidae, Baetidae and Simuliidae are more abundant  
337 in cascades.

338 At the reach scale, some of the indexes indicate significant differences. In particular,  
339 reaches differ in terms of Evenness indexes ( $p<0.05$ ), EPT abundance ( $p<0.05$ ) and  
340 percentage of Chironomidae ( $p<0.05$ ) (Figure 7). At the reach scale but within each  
341 segment, reaches A1, A2, and A3 showed quite low variance in richness values

(between 16 and 18 families). Reach A3 featured the highest diversity values ( $H=1.87$ ;  $1-D=0.77$ ), being reaches A1 ( $H=1.79$ ;  $1-D=0.76$ ) and A2 ( $H=1.86$ ;  $1-D=0.74$ ) lower in terms of diversity indexes (Figure 7). Within segment B, the reaches B1, B2, B3 presented the highest richness among the study site, ranging between 18 and 22 families, and featuring among the higher values of abundance (between 1007 and 608 specimens). However, these reaches were dominated by the Ephemeropterans *Baetidae* and Dipterans *Tipulidae*, thus having the lowest values of diversity (especially in terms of  $H$ ; Figure 7). Within segment C, the reach C1 had the highest abundance and richness ( $N=674$ ;  $S=17$ ), while C3 had the highest diversity index values ( $H=2.2$ ;  $1-D=0.87$ ), followed by C2 ( $H=1.82$ ;  $1-D=0.79$ ) and C1 ( $H=1.72$ ;  $1-D=0.72$ ) (Figure 7).

At the scale of the three river segments, segment B had the highest value of abundance and richness of macroinvertebrates ( $N=2405$ ;  $S=25$ ), followed by segment A ( $N=1601$ ;  $S=20$ ) and C ( $N=1268$ ;  $S=21$ ). There are significant differences in terms of Shannon-Wiener, Simpson and Evenness index, and the EPT and Chironomidae ( $p<0.05$ ), among the segments (Figure 8). The macroinvertebrates community in segment B was dominated by Ephemeropterans *Baetidae*, more precisely by *Andesiops torrens*. This resulted in lower biodiversity, represented by lower Shannon ( $H=1.5$ ), Simpson ( $1-D=0.66$ ) and Evenness indices ( $J=0.25$ ) (Figure 8). In segment A, higher values of the Shannon-Wiener ( $H=1.96$ ), Simpson ( $1-D=0.78$ ) and Evenness indices ( $J=0.42$ ) (Figure 8) showed a higher benthic macroinvertebrates diversity than in segment B. The highest diversity values were observed in segment C, where taxa displayed a more random distribution in terms of abundance and lower dominance, as showed by the Evenness ( $J=0.52$ ) and Simpson indices ( $1-D=0.84$ ). Further, a significant difference in the EPT representativeness between segments was observed, with lowest values for segment C (Figure 8).

### 3.4. Fish community at the scales of reach and segment.

Three fish species were identified in the Clarillo River, namely *Trichomycterus aerolatus* (Valenciennes, 1846), *Oncorhynchus mykiss* (Walbaum, 1792) and *Salmo trutta* (Linnaeus, 1758). The first is a Chilean species considered endemic, and widely distributed through central Chile (Habit *et al.*, 2006). It is typically found in habitats of cold flowing water and in sites with coarse sediments, although it can also be found

partially buried in fine sediments. *O. mykiss* and *S. trutta* are instead exotic introduced salmonid species (Habit *et al.*, 2006). Overall, *T. aerolatus* was the most abundant species (n=347), followed by *O. mykiss* (n=201) and *S. trutta fario* (n=14).

At the reach scale the highest abundance of specimens was found in reaches B1, B2 and B3, although no statistically significant differences were found ( $p>0.05$ ). *S. trutta* was found in small numbers but evenly distributed on the nine study reaches (Figure 9). *O. mykiss* was abundant in reaches B1, B2 and A3, and it was the dominant species in reaches A3, B1 and C2. *T. aerolatus* was abundant in reaches B1, B2, B3, C3, and it was the dominant species in reaches A2, B2, B3, C1 and C3 (Figure 9).

At the scale of segments, the abundance of fish varied, especially for *T. aerolatus* (Figure 9). In general, higher abundances were observed in segment B. Given the low number of species (S) observed, diversity indexes were not informative (data not showed).

The abundance of fish was well correlated with environmental variables measured at the scale of microhabitat and averaged at the scale of reach, such as averaged flow velocity ( $r=0.69$ ;  $p<0.05$ ), conductivity ( $r=0.72$ ;  $p<0.05$ ) and total dissolved solids (TDS,  $r=0.75$ ;  $p<0.05$ ) at the reach scale. More specifically, the mean flow velocity at the reach scale is well correlated with the abundance of *T. aerolatus* ( $r=0.73$ ;  $p<0.05$ ), the dissolved oxygen is well correlated with the abundance of *S. trutta* ( $r=0.67$ ;  $p<0.05$ ), and the TDS is well correlated with the abundance *O. mykiss* ( $r=0.76$ ;  $p<0.05$ ).

#### 3.4. Amphibian survey at the scales of reach and segment.

Two species were identified in the anuran group and a total of 54 specimens were found in the field. The most abundant species was *Pleuroderma thaul* (Lesson, 1826) (n=34) followed by *Rhinella aruncu* (Guichenot, 1848) (n=20), both being Chilean native species. Only *P. thaul* was observed in segment C, but both species were found in segments A and B, where *R. aruncu* was dominant (Figure 10). Segment C had the highest abundance (n=29), followed by segment B (n=14) and A (n=6) ( $p<0.05$ ; Figure 10). Significant differences in abundance of anurans were observed at the scale of segment but not between reaches ( $p<0.002$ ; Figure 10). At the reach scale, the mean elevation correlated negatively with abundance ( $r^2=0.64$ ,  $r=-0.8$ ;  $p<0.05$ ).

#### 3.5. Relationships between the diversity of geomorphic units and the biological diversity.

404 The strongest positive relationships between biological and geomorphic diversity were  
 405 found for the benthic macroinvertebrate group. In particular, the geomorphic unit density  
 406 within the bankfull channel (GUSI-D) showed a significant positive relationship with  
 407 Evenness diversity indexes ( $r=0.37$ ;  $r^2=0.14$ ;  $p<0.03$ ) (Figure 11a). The abundance of  
 408 geomorphic units relates mainly to the Evenness ( $r=0.86$ ,  $r^2=0.75$ ;  $p<0.005$ ), Simpson  
 409 ( $r=0.61$ ,  $r^2=0.37$ ;  $p<0.05$ ) and Shannon-Wiener indexes ( $r=0.55$ ,  $r^2=0.31$ ;  $p<0.05$ )  
 410 (Figure 11b and c). In addition, some specific geomorphic units tend to be associated  
 411 with certain biological metrics. For instance, there was an inverse relation between the  
 412 average area of pools (data not shown) and the Shannon-Weiner ( $r=-0.55$ ;  $r^2=0.3$ ;  
 413  $p<0.05$ ), Simpson ( $r=0.4$ ;  $r^2=-0.63$ ;  $p<0.05$ ), and Evenness indexes ( $r=0.34$ ;  $r^2=-0.59$ ;  
 414  $p<0.05$ ). On the other hand, there was a positive relation between the number of  
 415 cascades and Shannon ( $r=0.64$ ;  $r^2=0.41$ ;  $p<0.05$ ) and Simpson ( $r=0.55$ ;  $r^2=0.3$ ;  $p<0.05$ )  
 416 indexes (data not shown).  
 417 In terms of fish, the main relationship between geomorphic and biological diversity of  
 418 the studied river site was found between the density index of submerged geomorphic  
 419 units (mGUSI-D<sub>C</sub>) and the Margalef index D ( $r=0.61$ ,  $r^2=0.38$ ;  $p=0.0006$ ). Also, there  
 420 was a significant relationship between the number of pools and the Margalef diversity  
 421 index D ( $r=0.65$ ,  $r^2=0.42$ ;  $p=0.0003$ ), and between the number of steps and the Shannon  
 422 ( $r=0.64$ ;  $r^2=0.41$ ;  $p<0.05$ ) and Simpson indexes ( $r=0.50$ ;  $r^2=0.25$ ;  $p<0.007$ ) (Figure 12).  
 423 Furthermore, a significant relationship was found between the number of riffles and the  
 424 Shannon-Wiener index ( $r=0.48$ ,  $p<0.05$ ; data not shown). At the segment scale, we  
 425 found a positive (although not significant) relationship between the density index for the  
 426 submerged units and the Simpson diversity index and abundance of fish (except for *S.*  
 427 *trutta*). In terms of single species, *T. aerolatus* biomass is significantly correlated to the  
 428 average area of riffles ( $r=0.78$ ,  $r^2=0.62$ ;  $p<0.05$ ) and cascades ( $r=0.84$ ;  $r^2=0.7$ ;  $p<0.05$ )  
 429 (Figure 13). Besides, the biomass/SE of *O. wykiss* is significantly related to the mGUSI-  
 430 D<sub>C</sub> index ( $r=0.68$ ,  $r^2=0.46$ ;  $p<0.05$ ) (Figure 13), and the *S. trutta* is found in higher  
 431 abundance in reaches with more cascades ( $r^2=0.60$ ;  $r=0.77$ ,  $p=0.0137$ ).  
 432 Only few significant relationships were found between geomorphic diversity and  
 433 biological metrics for the amphibians group. The number of cascades correlated  
 434 negatively with the Shannon-Wiener and Simpson diversity indices ( $p<0.05$ ). Only a

not-statistically significant relation was found between diversity indexes and the number of types of geomorphic units and the geomorphic index of richness at the scale of segments.

#### **4. Discussion**

In the Clarillo River, a permanent near-natural and confined system in the Central Chilean Andes, we found some significant relationships between the geomorphological nature of the study site and the local biological communities. The three biological groups observed (macroinvertebrates, fish and anurans) responded differently to the geomorphological scale under consideration, confirming that the spatial scale is crucial in understanding the relationship between the physical and biological nature of the riverine ecosystem.

##### *4.1. Macroinvertebrates and the geomorphic units*

Large scale environmental conditions such as land use and physiochemical parameters at the basin scale are recognized to influencing the presence, abundance, and diversity of macroinvertebrates of a certain river site (e.g. Kaller and Hartmann, 2004; Leps *et al.*, 2015), but the local conditions also contribute to determine the composition of benthic macroinvertebrates communities (Harrisson *et al.*, 2007). Geomorphic diversity generally refers to the types and spatial distribution of geomorphic units in a certain area. However, the relationships between geomorphic and hydraulic units and the respective habitats for biota are very complex. Indeed, effects due to flow directionalities, zoogeomorphic footprint and ecological memory, add multiple levels of complexity to the relationship between morphological and biological diversity. Reid *et al.* (2010) reported that the heterogeneity of habitats influences macroinvertebrate communities in non-linear ways, and that habitat heterogeneity is likely to be related to greater diversity of macroinvertebrates. Our results show that, in the case of a near-natural confined system, the abundance of geomorphic units lead to a greater biological diversity of macroinvertebrates. The highest values of biological diversity metrics like the Shannon-Wiener (H), Simpson (1-D), and Evenness indices (J) were related to the reaches with highest abundance of geomorphic units. The latter is likely to support a greater habitat heterogeneity at micro-habitat scale, hence supporting greater diversity in

466 macroinvertebrate community.

467 The assemblages of species differed among geomorphic units with different depths and  
468 flow conditions. Overall, pools were the units with higher abundance and richness,  
469 resulting on higher diversity indexes. Through the relative habitat preference index  
470 (Sullivan et al., 2004), we observed an association between taxon at a geomorphic unit  
471 scale and within geomorphic unit types with slightly different hydraulic conditions (i.e.,  
472 slow or rapid flow and shallow or deep water). For example, specific families of  
473 dipterans (Athericidae and Ceratopogonidae), ephemeropterans (Leptophlaebidae),  
474 megaloptera (Hidrobiidae), coleopterans (Elmidae) and trichopterans (Glossosomatidae),  
475 were more dominating in pools, usually with slow flows and deeper water. On the other  
476 hand, trichopterans (Hydropsichidae), dipterans (Chironomidae) and oligochaeta  
477 (Lumbricidae) were mostly found in riffles, with shallow water and moderate flow  
478 velocity. The plecopterans (Gripopterygidae) and Odonata (Gripopterygidae) in this  
479 case, were associated to cascades.

480 Milner *et al.* (2015) stressed that the mosaic of different geomorphic types supports the  
481 “pool of species” and that a river composed of several geomorphic unit types provides  
482 more species than a river dominated by a single type. Interestingly, at the reach scale we  
483 observed that benthic diversity is well related with the number and density of  
484 geomorphic units rather than exclusively the different types of geomorphic units,  
485 suggesting that the complexity of the mosaic of geomorphic units to support biodiversity  
486 can also be determined by its patchiness (i.e. for a same number of types, a higher  
487 number of smaller units supports better biodiversity than few larger units). Indeed, given  
488 that the Clarillo River featured approximately the same geomorphic units in all study  
489 reaches, their relative abundance appears to be the main modulator of macroinvertebrate  
490 communities.

491 Pools are the smallest among the most abundant submerged geomorphic unit types, and  
492 were mainly related to abundance and richness of taxa. On the other hand, there were  
493 fewer riffles and cascades, but they were wider, longer and were also associated with  
494 abundance of macroinvertebrates. This suggests that also the relative size of specific  
495 geomorphic units is important for determining the range of macroinvertebrates. This  
496 relative size is likely associated with a ratio between the mean size of the unit and the



channel width, or as a ratio between the mean size of the unit and the grain size of sediments (Belletti *et al.*, 2017), and deserves further attention. In any case, it must be stressed that the abundance and richness of geomorphic units must be interpreted with care, as these units are not independent of each other from a morphological point of view, and also depend on the overall boundary conditions (Wheaton *et al.*, 2015; Belletti *et al.*, 2017). Similarly, the macroinvertebrate communities found on specific morphological units are not independent of communities found on surrounding morphological units or of upstream conditions (Frisell *et al.*, 1986).

At the larger scale of river segment, the Clarillo River showed significant differences in terms of macroinvertebrate diversity, although a longitudinal trend does not clearly emerge, as previously demonstrated by Thorp *et al.* (2006). Abundance and richness were higher in segment B, located in the middle segment of the studied river. In this segment, a taxonomic dominance is also occurring, leading to lower values of biodiversity indexes.

#### 4.2. Fish community and the morphological diversity at the reach scale

The fish community in the Clarillo River is composed of a small number of species and individuals. This was expected, since it is a small river and the fish community in Chile is limited to 44 native and a few exotic species (Habit *et al.*, 2006). The most abundant species in the Clarillo River was *T. aerolatus*, classified as an endemic species which overall indicates a good habitat status for the river ecosystem. The presence and abundance of *O. mykiss* and *S. trutta* is not necessarily a good indicator of excellent ecological conditions as both species are invasive.

Fish are key indicators of hydromorphological conditions due to their high mobility, physiology and ecological traits (e.g., Brey and Sullivan, 2015). Indeed, we could not determine a clear direct relationship between fish species and a preferred geomorphic unit. Also, it is unlikely that a single geomorphic unit could be preferred by a certain fish species for the entire life cycle (see also Schmutz *et al.*, 2014; Wolter *et al.*, 2016), as fish life-cycle depends on a shifting range of habitat mosaic at different spatial scales (Brennan *et al.*, 2019).

As for the diversity indexes of fish, the Margalef index was significantly correlated with the density index of submerged geomorphic units. Interestingly, some single species

528 were found in higher abundance on reaches with more specific morphological units (e.g.  
529 *S. trutta* and cascades). At this scale, the hydraulic nature of specific sites can determine  
530 the presence of different species (Eskew et al., 2012, Wolter *et al.* 2016; Grown *et al.*,  
531 2017). For instance, Bernardo et al (2003) showed that native species in rivers in Spain  
532 preferred shallow riffles whereas exotic species would prefer pools. In our study,  
533 reaches with larger numbers of morphological units with high velocity flows such as  
534 rapids and cascades featured higher abundance of fish, especially *T. aerolatus*. Instead,  
535 *O. mykiss* and *S. trutta* were found in reaches irrespective of their geomorphic units  
536 composition. This is likely due to the fact that geomorphic units have to be considered in  
537 the framework of how they are assembled with the surrounding units in complex  
538 patterns. It is also worth stressing that, to the best of our knowledge, there are virtually  
539 no studies which were able to determine the morphological preferences of native versus  
540 exotic freshwater species in Chile, and Chilean rivers are severely affected by presence  
541 of exotic species that are strongly affecting local endemic species (Rojas *et al.*, 2019).

542 The morphological complexity due to the spatial composition and density of geomorphic  
543 units also determines the diversity of fish. In our study, we found more fish on reaches  
544 with more riffles and cascades. However, in reaches with higher number of pools we  
545 found higher diversity. The fish diversity is better correlated with morphological  
546 diversity at an intermediate spatial size which is the reach scale, although fish abundance  
547 clearly depends on the habitats available in the whole hydrographic network (Schmutz et  
548 al., 2014; Brey and Sullivan, 2015; Wolter et al., 2016).

#### 549 *4.3. Amphibian community and the morphological diversity at the segment scale*

550 Anurans rely on both submerged and exposed geomorphic units at different stages of  
551 their life-cycle, and usually prefer habitats such as ephemeral ponds and nearshore  
552 channels sites. In the Clarillo River we could find no evidence of conclusive association  
553 between certain geomorphic units or river reaches, and biological characteristics or  
554 indexes for this small anuran community. However, we preferentially observed more  
555 anurans on reaches with more diverse morphological units in the small an elongated  
556 floodplain pockets (which were not surveyed using the GUS), as showed in previous  
557 studies (e.g., Reilly et al., 2015). A more detailed morphological survey on the  
558 floodplain could shed further light on the morphological characteristics of the

559 floodplains that these anurans tend to prefer. Also, more frequent field surveys could  
560 provide further evidence of abundance, since *P. thaul* is one of the most broadly  
561 distributed anurans in Chile (Díaz-Páez and Ortiz, 2003) and *R. aruncu* is the most  
562 abundant species in the Reserve and is considered a common species.

563 The Simpson index diversity was higher if calculated at the scale of segment, indicating  
564 that this is the spatial scale that is more likely to determine differences in the abundance  
565 of anurans. As argued by Tockner *et al.* (2006), amphibians are more strongly associated  
566 with the floodplain than with the main channel. In the island braided Tagliamento River  
567 (Italy), the active floodplains were found to be highly important habitats for amphibian  
568 distribution. In the study site, which is a confined river, direct observations suggest that  
569 anurans are more likely to be found in small pools along the gravel bars or in portions of  
570 the active channel with low flow velocity.

#### 571 *4.4. Relationships between geomorphic and biological diversity*

572 The three groups of organisms studied in the current investigation are considered good  
573 indicators of environmental conditions. Fish are indicators for aquatic habitats  
574 conditions, such as the rheophilic fish species which have a significant response to river  
575 changes (Eskew *et al.*, 2012; Schmutz *et al.*, 2014). Amphibians and macroinvertebrates  
576 are indicators of both aquatic and fluvial terrestrial environments, as they occupy  
577 different types of habitats over the entire fluvial corridor during their life cycles.

578 In the Clarillo River, the abundance and diversity of macroinvertebrates were more  
579 closely related with the abundance and density of geomorphic units, whereas the  
580 abundance of fish related to a higher degree with the reach and segment scales, and the  
581 amphibians were more broadly related to the nature of the river segments. This  
582 reinforces the idea that geomorphic influence is part of a larger chain of events in a  
583 hierarchical spatio-temporal scale that sustains such environmental complexity (Frissel  
584 *et al.*, 1986; Amoros & Petts, 1993; Friberg *et al.*, 2009; Belletti *et al.*, 2017; Leps *et al.*  
585 2015). The results of this study stress that both biological and geomorphic diversity and  
586 their interactions need to be considered at a variety of scales, and that the degree to  
587 which they relate depends on what is measured. Indeed, as pointed out by Fryirs &  
588 Brierley (2009), promoting geomorphic diversity at the reach scale (which is usually the  
589 scale at which river restoration projects are implemented) is not necessarily appropriate

for each site and may not result in higher diversity of the targeted biota. Also, as pointed out by Brennan *et al.* (2019) the habitat mosaics supporting biota should be considered dynamic in time (i.e., shifting due to interactions with geomorphological processes and biological responses) and at the scale of the entire watershed. Furthermore, the hydrological regime and the geological characteristics of the basin should be taken into account to understand how biology interact with fluvial geomorphology (Castro & Thorne, 2019). Additionally, we did not consider biological interactions between biota at different levels in the riverine environment (i.e., competition and predation) that could also affect considerably the outcomes of this investigation.

The current study compared a considerable group of environmental and biological variables at multiple spatial scales. However, only a few very significant statistical differences were observed, probably because the reaches belong to similar river sections, which have very comparable intrinsic morphological conditions and negligible human pressures. Though, the present study expands our knowledge of the current eco-hydromorphology for the upper part of the Clarillo River. The extensive field survey provided a “snapshot” about the geomorphic and biological nature of the studied river section. Further efforts in this direction should focus on exploring fluvial processes (e.g. flow and sediment regimes and channel changes) rather than only forms, and to monitor seasonal changes of aquatic communities in order to include a temporal component in the analysis, and to allow for future habitat modeling efforts in relating geomorphic and biological diversity (e.g. Vezza *et al.*, 2014). Indeed, natural fluvial process triggered by floods can increase geomorphic diversity (Williams *et al.*, 2020), and this can affect the diversity of biota in different ways depending on the magnitude and seasonality of the events, and on the recovery of the system within the long-term evolutionary trajectory of the river system (Brierley & Fryirs, 2005), which is worth exploring as a whole.

#### 4.5. Potential implications

Chilean rivers are particularly sensitive systems, given their peculiar natural condition of isolation in South America (i.e., basins are constrained by the Andes and the Pacific Ocean, and are generally short and steep), which sustains a low level of biodiversity, when compared to rivers in other countries of the continent. However, the same conditions contribute to a high level of endemism (Rojas *et al.*, 2019). For instance,

among the different bioclimatic regions (i.e. arid to humid), the Mediterranean zone is a hotspot for biodiversity, sustaining an important part of Chilean fauna (i.e. higher values of richness for fish species). Unfortunately, the Mediterranean zone hosts large urban areas, intensive agriculture and mining sites, all of which is threatening the functioning and biodiversity of Chilean rivers (i.e., water overexploitation, intense gravel mining, decrease of water quality). In particular, unfortunate management choices and excessive exploitation of river services are leading to a very threatening context (Andreoli et al., 2012). Although there are increasing more studies concerning river management in Chile, there are still no examples of river restoration being implemented. The current study highlights the importance of considering biodiversity-geodiversity interactions at the right scale for different purposes. Furthermore, as recently pointed out by Johnson *et al.* (2020), in case of river restoration the biota should also be recognized as an active factor that can determine fluvial forms and processes. Our work can help selecting the right scale for sampling and monitoring in Environmental Impact Assessment of river-related projects in Chile, which are currently under-standard in Chile, especially for fish (Lacy et al. 2017). In this sense, wider and proactive sampling and monitoring programs carried out at the regional/national scale would represent a critical baseline to appraise the conditions of Chilean rivers and understand natural and anthropogenic controls upon future trajectories of change. More specifically, being a protected site, the dataset collected in the Clarillo River could allow future assessments of the ecological consequences of evolutionary trajectories of the river morphological and biological adaptations to climate change, that in the area is characterized by a decade-long period of precipitation deficit (e.g., Alvarez-Garreton et al., 2020).

644

## 645 **5. Final remarks**

Our study highlights the relationship between geomorphological and biological diversity, where the availability and diversity of morphological units is the key driver for biodiversity. Our results suggest that availability of habitats provides the conditions to support different levels of biodiversity in a hierarchical way. In particular, we found that abundance and diversity of macroinvertebrates are more related to the abundance of geomorphic units, whereas the presence of fish is more related to the geomorphic nature

652 of the reaches, and the presence of anurans is more related to the nature of river  
653 segments.

654 This study reinforces the need to expand the field survey protocols and assessments to a  
655 broader scale, as suggested by others (i.e., Wolter et al., 2016). The MQI and the GUS  
656 protocols adapted in this study are termed multiscale since their approaches range from  
657 basins to geomorphic unit scales (Rinaldi et al., 2015; 2017). The approach of including  
658 topographic data in research focusing the geo-biological relationship is not very  
659 common and it can be crucial for the ecological riverine studies in a near future (Kärnä  
660 *et al.*, 2019). Here we combined these protocols (MQI and GUS) with biological surveys  
661 as an attempt to incorporate geomorphological conditions at different scales and  
662 biological composition, for the first time in Latin America. The use of the hierarchical  
663 approach ranging from the basin to the geomorphic unit scales should be taken as a  
664 necessary input to improve the existing assessment tools and to support future  
665 management or conservations plans from the local to the regional scale in the Chilean  
666 territory.

667

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673

## 674 **List of references**

- 675 Alarcon, V. J., H. Alcayaga, and E. Álvarez. 2015. Hydrological modeling of an  
676 ungauged watershed in Southern Andes. AIP Conference Proceedings 1702,  
677 190003. <https://doi.org/10.1063/1.4938970>
- 678 Alvarez-Garreton, C., J. P. Boisier, R. Garreaud, J. Seibert, and M. Vis. in review.  
679 Progressive water deficits during multi-year droughts in central-south Chile,  
680 Hydrology and Earth System Sciences Discussion, [https://doi.org/10.5194/hess-](https://doi.org/10.5194/hess-2020-249)  
681 2020-249.
- 682 Andreoli, A., L. Mao, A. Iroume, J. Arumi, A. Nardini, R. Pizarro, D. Caamaño, C.

683 Meier, and O. Link. 2012. The need for a hydromorphological approach to  
684 Chilean river management. *Revista Chilena de Historia Natural* 85:339-343.

685 Belletti, B., M. Rinaldi, A. D. Buijse, A. M. Gurnell, and E. Mosselman. 2015. A review  
686 of assessment methods for river hydromorphology. *Environmental Earth*  
687 *Sciences* 73(5):2079-2100.

688 Belletti, B., M. Rinaldi, M. Bussetini, F. Comiti, A. M. Gurnell, L. Mao, L. Nardi, and  
689 P. Vezza. 2017. Characterising physical habitats and fluvial hydromorphology: A  
690 new system for the survey and classification of river geomorphic units.  
691 *Geomorphology* 283:143-157.

692 Bernardo, J. M., M. Ilhéu, P. Matono, and A. M. Costa. 2003. Interannual variation of  
693 the fish assemblage structure in a mediterranean river: implications of  
694 streamflow on the dominance of native or exotic species. *River Research and*  
695 *Applications* 19:521-532.

696 Brennan, S. R., D. E. Schindler, T. J. Cline, T. E. Walsworth, G. Buck, and D. P.  
697 Fernandez. 2019. Shifting habitat mosaics and fish production across river  
698 basins. *Science* 364:783-786.

699 Brey, C. R., and S. M. P. Sullivan. 2015. Associations between stream  
700 hydrogeomorphology and co-dependent mussel-fish assemblages: Evidence from  
701 an Ohio, USA river system. *Aquatic Conservation: Marine and Freshwater*  
702 *Ecosystems* 25(4):555-568.

703 Brierley, G., C. Cullum, M. Tadaki, and B. Blue. 2013. Reading the landscape:  
704 Integrating the theory and practice of geomorphology to develop place-based  
705 understandings of river systems. *Progress in Physical Geography* 37(5):601-621.

706 Castro, J. M., and C. R. Thorne. 2019. The stream evolution triangle: Integrating  
707 geology, hydrology, and biology. *River Research and Applications* 35(4):315-  
708 326.

709 Chessman, B. C., K. A. Fryirs, and G. J. Brierley. 2006. Linking geomorphic character,  
710 behaviour and condition to fluvial biodiversity: implications for river  
711 management. *Aquatic Conservation: Marine and Freshwater Ecosystems*,  
712 16(3):267-288.

713 Díaz-Páez, H., and J. C. Ortiz. 2003. Assessment of the conservation status of

714           amphibians in Chile. *Revista Chilena de Historia Natural* 76(3):509-525.

715   Eskew, E. A., S. J. Price, and M. E. Dorcas. 2012. Effects of River-Flow Regulation on  
716           Anuran Occupancy and Abundance in Riparian Zones. *Conservation Biology*  
717           26(3):504-512.

718   Friberg, N., L. Sandin, and M. L. Pedersen. 2009. Assessing the effects of  
719           hydromorphological degradation on macroinvertebrate indicators in rivers:  
720           examples, constraints, and outlook. *Integrated Environmental Assessment and*  
721           *Management* 5(1):86-96.

722   Frissell, C. A., W. J. Liss, C. E. Warren, and M. D. Hurley 1986. A hierarchical  
723           framework for stream habitat classification: Viewing streams in a watershed  
724           context. *Environmental Management* 10(2):199-214.

725   Fryirs, K. A., and G. J. Brierley. 2013. *Geomorphic analysis of river systems: an*  
726           *approach to reading the landscape*. John Wiley & Sons.

727   Fryirs, K., and G. J. Brierley. 2009. Naturalness and place in river rehabilitation.  
728           *Ecology and Society* 14(1):20.

729   Golfieri, B., N. Surian, and S. Hardersen. 2015. Towards a more comprehensive  
730           assessment of river ecological conditions: A comparison between the  
731           Morphological Quality Index and three biotic indices. *Ecological indicators*  
732           84:525-534.

733   Growns, I., J. F. Murphy, and J. I. Jones. 2017. The effects of altered flow and bed  
734           sediment on macroinvertebrates in stream mesocosms. *Marine and Freshwater*  
735           *Research* 68(3):496-505.

736   Gurnell, A. M., M. Rinaldi, B. Belletti, S. Bizzi, B. Blamauer, G. Braca, A. D. Buijse,  
737           M. Bussetini, B. Camenen, F. Comiti, L. Demarchi, D. García de Jalón, M.  
738           González del Tánago, R. C. Grabowski, I. D. M. Gunn, H. Habersack, D.  
739           Hendriks, A. J. Henshaw, M. Klösch, B. Lastoria, A. Latapie, P. Marcinkowski,  
740           V. Martínez-Fernández, E. Mosselman, J. O. Mountford, L. Nardi, T. Okruszko,  
741           M. T. O'Hare, M. Palma, C. Percopo, N. Surian, W. van de Bund, C.  
742           Weissteiner, and L. Ziliani. 2016. A multi-scale hierarchical framework for  
743           developing understanding of river behaviour to support river management.  
744           *Aquatic Sciences* 78(1):1-16.



- Habit, E., B. Dyer, and I. Vila. 2006. Current State of Knowledge of Freshwater Fishes of Chile. *Gayana* 70(1):100-113.
- Harrison, S. S. C., J. L. Pretty, D. Shepherd, A. G. Hildrew, C. Smith, and R. D. Hey. 2004. The effect of instream rehabilitation structures on macroinvertebrates in lowland rivers. *Journal of Applied Ecology* 41(6):1140-1154.
- Johnson, M. F., C. R. Thorne, J. M. Castro, G. M. Kondolf, C. S. Mazzacano, S. B. Rood, and C. Westbrook. 2020. Biomic river restoration: A new focus for river management. *River Research and Applications*, 36:3-12.
- Junk, W. J., P. B. Bayley, and R. E. Sparks. 1989. The flood pulse concept in river-floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences* 106(1):110-127.
- Kaller, M. D. D., and K. J. J. Hartman. 2004. Evidence of a threshold level of fine sediment accumulation for altering benthic macroinvertebrate communities. *Hydrobiologia* 518(1):95-104.
- Kärnä, O-M., J. Heino, T. Laamanen, J. Jyrkänkallio-Mikkola, V. Pajunen, J. Soininen, K. T. Tolonen, H. Tukiainen, and J. Hjort. 2019. Does catchment geodiversity foster stream biodiversity? *Landscape Ecology* 34:2469-2485.
- Lacy, S., F. J. Meza, and P. A. Marquet. 2017. Can environmental impact assessments alone conserve freshwater fish biota? Review of the Chilean experience. *Environmental Impact Assessment Review* 63:87-94.
- Leps, M., J. D. Tonkin, V. Dahm, P. Haase, and A. Sundermann. 2015. Disentangling environmental drivers of benthic invertebrate assemblages: The role of spatial scale and riverscape heterogeneity in a multiple stressor environment. *Science of the Total Environment* 536:546-556.
- Maddock, I. 1999. The importance of physical habitat assessment for evaluating river health. *Freshwater Biology*, 41(2):373-391.
- Milner, V. S., N. J. Willby, D. J. Gilvear, and C. Perfect. 2015. Linkages between reach-scale physical habitat and invertebrate assemblages in upland streams. *Marine and Freshwater Research* 66:438-448.
- Palmer, M. A., H. L. Menninger, and E. Bernhardt. 2010. River restoration, habitat heterogeneity and biodiversity: a failure of theory or practice? *Freshwater*

776 Biology, 55:205-222.

777 Palmer, M., and A. Ruhi. 2019. Linkages between flow regime, biota, and ecosystem  
778 processes: Implications for river restoration. *Science*, 365,6459:eaaw2087. DOI:  
779 10.1126/science.aaw2087.

780 Poeppel, R. E., Fryirs, K. A., Tunnicliffe, J., and G. J. Brierley. 2020. Managing sediment  
781 (dis)connectivity in fluvial systems. *Science of the Total Environment*  
782 736:139627.

783 Poole, G. C. 2010. Stream hydrogeomorphology as a physical science basis for advances  
784 in stream ecology. *Journal of the North American Benthological Society*,  
785 29(1):12-25.

786 Rasmussen, J. J., A. Baattrup-pedersen, R. Tenna, and F. Nikolai. 2011. Stream  
787 ecosystem properties and processes along a temperature gradient. *Aquatic*  
788 *Ecology* 45:231-242.

789 Reid, H. E., G. J. Brierley, and I. K. G. Boothroyd. 2010. Influence of bed heterogeneity  
790 and habitat type on macroinvertebrate uptake in peri-urban streams. *International*  
791 *Journal of Sediment Research*, 25(3):203-220.

792 Reilly, S., R. Essner, S. Wren, L. Easton, and P. J. Bishop. 2015. Movement patterns in  
793 leiopelmatid frogs: Insights into the locomotor repertoire of basal anurans.  
794 *Behavioural Processes* 121:43-53.

795 Rinaldi, M., B. Belletti, M. Bussetini, F. Comiti, B. Gol, B. Lastoria, E. Marchese, L.  
796 Nardi, and N. Surian. 2017. New tools for the hydromorphological assessment  
797 and monitoring of European streams. *Journal of Environmental Management*  
798 202(2):363-378.

799 Rinaldi, M., N. Surian, F. Comiti, and M. Bussetini. 2015. A methodological  
800 framework for hydromorphological assessment, analysis and monitoring  
801 (IDRAIM) aimed at promoting integrated river management. *Geomorphology*  
802 251:122-136.

803 Rojas P., I. Vila E. Habit, and S. A. Castro. 2019. Homogenization of the freshwater fish  
804 fauna of the biogeographic regions of Chile. *Global Ecology and Conservation*  
805 19, e00658.

806 Schmutz, S., H. Kremser, A. Melcher, M. Jungwirth, S. Muhar, H. Waidbacher, and G.

807           Zauner. 2014. Ecological effects of rehabilitation measures at the Austrian  
808           Danube: a meta-analysis of fish assemblages. *Hydrobiologia* 729:49-60.

809   Schmutz, S., P. Jurajda, S. Kaufmann, A. W. Lorenz, S. Muhar, A. Paillex, M. Poppe,  
810           and C. Wolter. 2016. Response of fish assemblages to hydromorphological  
811           restoration in central and northern European rivers. *Hydrobiologia* 769(1):67-78.

812   Sullivan, S. M. P., M. C. Watzin, and W. C. Hession. 2004. Understanding stream  
813           geomorphic state in relation to ecological integrity evidence using habitat  
814           assessments and macroinvertebrates. *Environmental Management* 34:669-683.

815   Thomson, J. R., M. P., Taylor, and G. J. Brierley. 2004. Are River Styles ecologically  
816           meaningful? A test of the ecological significance of a geomorphic river  
817           characterization scheme. *Aquatic Conservation: Marine and Freshwater*  
818           *Ecosystems*, 14(1): 25-48.

819   Thorp, J. H., M. C. Thoms, and M. D. Delong. 2006. The riverine ecosystem synthesis:  
820           biocomplexity in river networks across space and time. *River Research and*  
821           *Applications* 22(2):123-147

822   Tockner K., I. Klaus, C. Baumgartner, and J. V. Ward. 2006. Amphibian Diversity and  
823           Nestedness in a Dynamic Floodplain River (Tagliamento, NE-Italy).  
824           *Hydrobiologia* 565(1):121-133.

825   Tockner, K., F. Malard, and J. V. Ward. 2010. An extension of the flood pulse concept.  
826           *Hydrological Processes*. 14(16- 17), 2861-2883.

827

828

829   Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980.  
830           The river continuum concept. *Canadian journal of fisheries and aquatic sciences*  
831           37:130-137.

832   Vezza, P., P. Parasiewicz, M. Spairani, and C. Comoglio. 2014. Habitat modeling in  
833           high-gradient streams: The mesoscale approach and application. *Ecological*  
834           *Applications* 24(4):844-861.

835   Wheaton, J. M., K. A. Fryirs, G. Brierley, S. G. Bangen, N. Bouwes, and G. O'Brien.  
836           2015. Geomorphic mapping and taxonomy of fluvial landforms. *Geomorphology*  
837           248:273-295.

838 Williams R. D., S. Bangen, E. Gillies, N. Kramer, H. Moir, J. Wheaton. 2020. Let the  
839 river erode! Enabling lateral migration increases geomorphic unit diversity.  
840 Science of The Total Environment 715:136817.

841 Wohl, E. 2014. Rivers in the landscape: science and management. Wiley-  
842 Blackwell

843 Wohl, E., S. N. Lane, and A. C. Wilcox. 2015. The science and practice of river  
844 restoration. Water Resources Research 51(8):5974-5997.

845 Wolter, C., A. D. Buijse, and P. Parasiewicz. 2016. Temporal and spatial patterns of fish  
846 response to hydromorphological processes. River Research and application  
847 32(2):190-201.

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869 **TABLES**

870 **Table 1.** The surveyed reaches on the Clarillo River.

| Reach | Length<br>(m) | Basin area<br>(Km <sup>2</sup> ) | Max.<br>elevation<br>(m a.s.l.) | Min.<br>elevation<br>(m a.s.l.) | Slope<br>(m/m) | Mean<br>bankfull<br>width (m) | D <sub>50</sub> (mm) | Dominant<br>morphology |
|-------|---------------|----------------------------------|---------------------------------|---------------------------------|----------------|-------------------------------|----------------------|------------------------|
| A1    | 230           | 1.7                              | 1101                            | 1085                            | 0.06           | 8.29                          | 47.77                | Step-cascade           |
| A2    | 160           | 1.33                             | 1139                            | 1071                            | 0.12           | 9.19                          | 40.56                | Riffle-cascade         |
| A3    | 170           | 1.2                              | 1062                            | 1038                            | 0.17           | 7.8                           | 34.62                | Riffle-cascade         |
| B1    | 130           | 1.45                             | 994                             | 991                             | 0.02           | 8.63                          | 33.56                | Riffle-cascade         |
| B2    | 230           | 1.29                             | 991                             | 979                             | 0.08           | 9.17                          | 33.2                 | Riffle-cascade         |
| B3    | 140           | 1.05                             | 977                             | 972                             | 0.03           | 10.37                         | 35.77                | Cascade                |
| C1    | 211           | 1.5                              | 885                             | 875                             | 0.04           | 10.5                          | 15.18                | Cascade                |
| C2    | 230           | 1.9                              | 876                             | 870                             | 0.03           | 9.48                          | 14.52                | Riffle-cascade         |
| C3    | 206           | 2.4                              | 830                             | 814                             | 0.09           | 11.2                          | 28.8                 | Riffle-Cascade         |

871 **Table 2.** Characteristics and MQI values of the Clarillo River segments along the entire  
872 network. The detailed morphological and ecological surveys were conducted on segment  
873 8 (A), 7 (B), and 4 (C).

| Segmen<br>ts | Length<br>(km) | Max.<br>elevation<br>(m a.s.l.) | Min.<br>elevation<br>(m a.s.l.) | Slope<br>(m/ml-l) | Mean<br>bankfull<br>width<br>(m) | Confinement<br>index | Sinuosity<br>index | MQI<br>index |
|--------------|----------------|---------------------------------|---------------------------------|-------------------|----------------------------------|----------------------|--------------------|--------------|
| 1            | 2.3            | 636                             | 609                             | 0.01              | 15.40                            | 2.6                  | 1.4                | 0.6          |
| 2            | 5.7            | 666                             | 637                             | 0.05              | 9.53                             | 2.6                  | 1.35               | 0.62         |
| 3            | 5.4            | 743                             | 669                             | 0.01              | 28.33                            | 2.8                  | 1.07               | 0.6          |
| 4 = C        | 3.8            | 843                             | 743                             | 0.02              | 17.00                            | 2                    | 1.16               | 0.7          |
| 5            | 2.0            | 903                             | 842                             | 0.06              | 10.70                            | 1.9                  | 1.2                | 0.89         |
| 6            | 1.7            | 946                             | 904                             | 0.07              | 9.25                             | 1.3                  | 1.3                | 0.93         |
| 7 = B        | 3.16           | 1032                            | 948                             | 0.03              | 12.97                            | 1.9                  | 1.3                | 0.94         |
| 8 = A        | 2.8            | 1148                            | 1029                            | 0.05              | 12.07                            | 1.7                  | 1.3                | 0.94         |
| 9            | 1.0            | 1213                            | 1149                            | 0.07              | 8.56                             | 1.5                  | 1.2                | 0.97         |
| 10           | 3.05           | 1415                            | 1208                            | 0.06              | 10.00                            | <1.5                 | 1.3                | 0.94         |
| 11           | 2.9            | 1663                            | 1414                            | 0.07              | 13.87                            | <1.5                 | 1.2                | 0.92         |
| 12           | 1.53           | 1778                            | 1662                            | 0.06              | 10.57                            | 1.5                  | 1.3                | 0.95         |
| 13           | 2.6            | 2170                            | 1773                            | 0.15              | 9.97                             | <1.5                 | 1.3                | 0.92         |
| 14           | 3.45           | 1468                            | 1148                            | 0.09              | 12.03                            | <1.5                 | 1.3                | 0.97         |
| 15           | 2.0            | 1679                            | 1466                            | 0.11              | 11.03                            | <1.5                 | 1.2                | 0.95         |
| 16           | 3.26           | 2006                            | 1680                            | 0.12              | 10.63                            | <1.5                 | 1.4                | 0.95         |
| 17           | 2.2            | 2189                            | 2007                            | 0.08              | 10.17                            | <1.5                 | 1.3                | 0.9          |
| 18           | 2.06           | 2149                            | 1779                            | 0.2               | 11.20                            | <1.5                 | 1.2                | 0.9          |
| 19           | 3.0            | 1664                            | 1061                            | 0.07              | 12.43                            | <1.5                 | 1.3                | 0.47         |
| 20           | 2.9            | 1796                            | 1156                            | 0.21              | 10.53                            | <1.5                 | 1.1                | 0.49         |
| 21           | 3.5            | 670                             | 653                             | 0.004             | 15.20                            | 1.5                  | 1.4                | 0.75         |

**Table 3.** Results of the application of GUS at the scale of reach.  $\sum NT_{GU}$ : number of types of geomorphic units;  $\sum N_{GU}$ : number of geomorphic units; NSteps: number of steps; NPools: number of pools; NRiffles: number of riffles; NCascade: number of cascades; NSubm: number of submerged units; %Steps: proportion of steps as submerged morphological units; %Pools: proportion of pools as submerged morphological units; %Riffles: proportion of riffles as submerged morphological units; %Cascade: proportion of cascades as submerged morphological units; Steps: area represented by steps within the bankfull (%); Pools: area represented by pool within the bankfull (%); Riffles: area represented by riffles within the bankfull (%); Cascades: area represented by cascades within the bankfull (%); Submerged area: area represented by submerged morphological units within the bankfull (%); Emerged areas: area represented by emerged morphological units within the bankfull (%); mGUSI- $R_{BC}$ : modified index of geomorphic unit richness for bankfull channel units; GUSI-D: linear density index of geomorphic units (n/km); mGUSI-D\_A: modified density index of geomorphic units (areal density); GUSI- $R_C$ : a richness index for submerged units; mGUSI-D $_C$ : modified sub-index of density of submerged units (number of submerged GU/reach length: m); GUSI-D $_C$ : density index of submerged units (n/km<sup>2</sup>); GUSI-D $_E$ : density index of emerging geomorphic units (n/km<sup>2</sup>).

| Reach          | A1   | A2   | A3   | B1   | B2   | B3   | C1   | C2   | C3   |
|----------------|------|------|------|------|------|------|------|------|------|
| $\sum NT_{GU}$ | 7    | 7    | 7    | 8    | 8    | 8    | 8    | 8    | 10   |
| $\sum N_{GU}$  | 63   | 44   | 62   | 49   | 44   | 45   | 75   | 82   | 62   |
| NPools         | 14   | 10   | 12   | 14   | 11   | 12   | 14   | 21   | 7    |
| NSteps         | 14   | 6    | 6    | 5    | 9    | 11   | 6    | 13   | 7    |
| NRiffles       | 3    | 2    | 4    | 3    | 1    | 2    | 6    | 3    | 3    |
| NCascade       | 4    | 4    | 9    | 4    | 1    | 4    | 5    | 3    | 6    |
| NSubm          | 35   | 22   | 31   | 26   | 22   | 29   | 31   | 40   | 23   |
| %Steps (n)     | 0.40 | 0.27 | 0.19 | 0.19 | 0.41 | 0.38 | 0.19 | 0.33 | 0.30 |
| %Pools (n)     | 0.40 | 0.45 | 0.39 | 0.54 | 0.50 | 0.41 | 0.45 | 0.53 | 0.30 |
| %Riffles (n)   | 0.09 | 0.09 | 0.13 | 0.12 | 0.05 | 0.07 | 0.19 | 0.08 | 0.13 |
| %Cascade (n)   | 0.11 | 0.18 | 0.29 | 0.15 | 0.05 | 0.14 | 0.16 | 0.08 | 0.26 |
| Steps (% area) | 0.38 | 0.02 | 0.30 | 0.08 | 0    | 0.15 | 0.02 | 0.01 | 0    |
| Pools (% area) | 0.11 | 0.01 | 0.01 | 0.02 | 0    | 0.14 | 0    | 0.01 | 0    |

|                         |        |      |        |        |        |        |        |        |      |
|-------------------------|--------|------|--------|--------|--------|--------|--------|--------|------|
| Riffles (% area)        | 0.12   | 0.15 | 0.22   | 0.34   | 0.34   | 0.15   | 0.27   | 0.18   | 0.28 |
| Cascades (% area)       | 0.17   | 0.49 | 0.21   | 0.47   | 0.53   | 0.47   | 0.51   | 0.47   | 0.42 |
| Submerged area (% area) | 0.78   | 0.67 | 0.74   | 0.91   | 0.87   | 0.91   | 0.80   | 0.67   | 0.70 |
| Emerged area (% area)   | 0.22   | 0.33 | 0.26   | 0.09   | 0.13   | 0.09   | 0.20   | 0.33   | 0.30 |
| mGUSI-R <sub>BC</sub>   | 0.28   | 0.28 | 0.28   | 0.32   | 0.32   | 0.32   | 0.32   | 0.32   | 0.32 |
| GUSI-D                  | 273.91 | 275  | 364.71 | 376.92 | 191.30 | 321.43 | 357.14 | 356.52 | 310  |
| mGUSI-D_A               | 0.03   | 0.03 | 0.05   | 0.03   | 0.03   | 0.03   | 0.04   | 0.03   | 0.02 |
| GUSI-R <sub>C</sub>     | 0.5    | 0.5  | 0.5    | 0.5    | 0.5    | 0.8    | 0.5    | 0.5    | 0.5  |
| mGUSI-D <sub>C</sub>    | 0.16   | 0.14 | 0.19   | 0.21   | 0.1    | 0.21   | 0.15   | 0.18   | 0.12 |
| GUSI-D <sub>C</sub>     | 0.03   | 0.02 | 0.033  | 0.02   | 0.02   | 0.021  | 0.02   | 0.02   | 0.01 |
| GUSI-D <sub>E</sub>     | 0.06   | 0.04 | 0.084  | 0.06   | 0.08   | 0.069  | 0.21   | 0.08   | 0.04 |

## FIGURE CAPTION

**Figure 1.** Location and map of the Clarillo River basin, and the boundaries of the Natural Reserve of Clarillo River (in green). The segments of the river network are colored reflecting their hydromorphological condition assessed using the MQI method proposed by Rinaldi *et al.* (2015) (blue: high; yellow: good; orange: moderate). The yellow lines indicate the study segments, corresponding to A, B and C (from upstream to downstream).

**Figure 2.** Synthetic representation of the range of spatial scales at which morphological and biological samplings and analysis were performed.

**Figure 3.** Pictures of the most common geomorphic units in the Clarillo River: a) riffle; b) cascade; c) step; and d) pool.

**Figure 4.** Pictures of the biological field samplings: a) macroinvertebrate sampling using a Surber sampler; b) fish capture using the electrofishing technique; c) a specimen of *O. mykiss* being measured; and d) a specimen of *R. aruncu* being captured during night visual encounter surveys.

**Figure 5.** Example of the application of the GUS (Belletti *et al.*, 2017) for the survey and evaluation of meso-habitats for the reach C2 (close-range drone photo on the left

and GUS application on the right). The geomorphic units showed here are: F: Riparian Zone; LB: Lateral bar; MCB: Mid-channel bar; RS: rock step; P: pool; I: island, R: riffle, and C: cascade.

**Figure 6.** Differences in abundance N (a) and richness s (b) of benthic macroinvertebrates at the scale of geomorphic unit. The solid line indicates the range between the 25<sup>th</sup> and 75<sup>th</sup> percentiles, the square indicates the median, the whiskers indicate the maximum and minimum non-outlier values. Solid circles indicate outliers and diamonds indicate extreme values. Difference was evaluated using an ANOVA test.

**Figure 7.** Differences in Shannon-Wiener (H) and Simpson diversity index (1-D) (a), Evenness (J) and Margaleff index (D) (b) and indexes of Ephemeroptera Trichoptera Plecoptera (ETP) and Chironomids indicators (c) for benthic macroinvertebrates at the reach scale (A1 to C3). The solid line indicates the range between the 25<sup>th</sup> and 75<sup>th</sup> percentiles, the square indicates the median, the whiskers indicate the maximum and minimum non-outlier values, the solid circles indicate outliers and the diamonds indicate extreme values. Difference was evaluated using an ANOVA test.

**Figure 8.** Differences in Shannon-Wiener (H) and Simpson diversity index (1-D) (a), Evenness (J) and Margaleff index (M) (b) and representability of Ephemeroptera Trichoptera Plecoptera (ETP) and Chironomid indicators (c) for benthic macroinvertebrates at the segment scale (A, B, C). The solid line indicates the range between the 25<sup>th</sup> and 75<sup>th</sup> percentiles, the square indicates the median, the whiskers indicate the maximum and minimum non-outlier values, the solid circles indicate outliers and the diamonds indicate extreme values. Difference was evaluated using an ANOVA test.

**Figure 9.** Differences in fish abundance (a) and values of capture per unit of effort (b) at the reach scale (A1 to C3) and differences of fish abundance at the segment scale (c). The solid dark line indicates the species *T. aerolatus*, the solid blue line indicates the *O.mykiss* and the dashed line indicates *S.trutta*. Difference was evaluated using an ANOVA test and Kruskal-Wallis test.

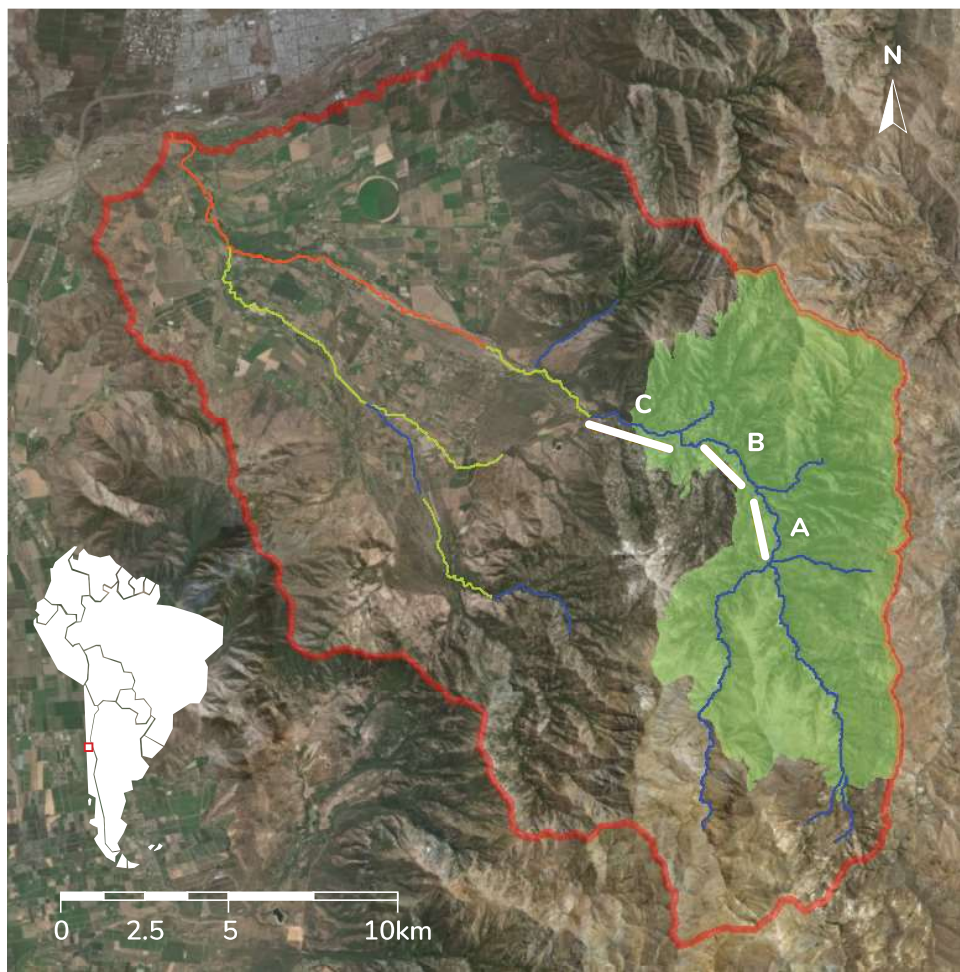
**Figure 10.** Differences in anuran abundance at the reach (a) and segment (b) scales. The solid dark line indicates the species *P thaul*, the solid grey line indicates the *R. aruncu*. Difference was evaluated using an ANOVA test and Kruskal-Wallis test.







942 **Figure 11:** Correlations between geomorphic units index or values and ecological  
943 indexes for the macroinvertebrate community: a) GUSI-D density index vs. Evenness  
944 (J); b) Number of Geomorphic Units vs. Evenness index; c) Number of Geomorphic  
945 Units vs Shannon-Wiener (H) and Simpson diversity index (1-D). The correlation  
946 analysis was performed using the Pearson correlation at  $p < 0.05$ ).

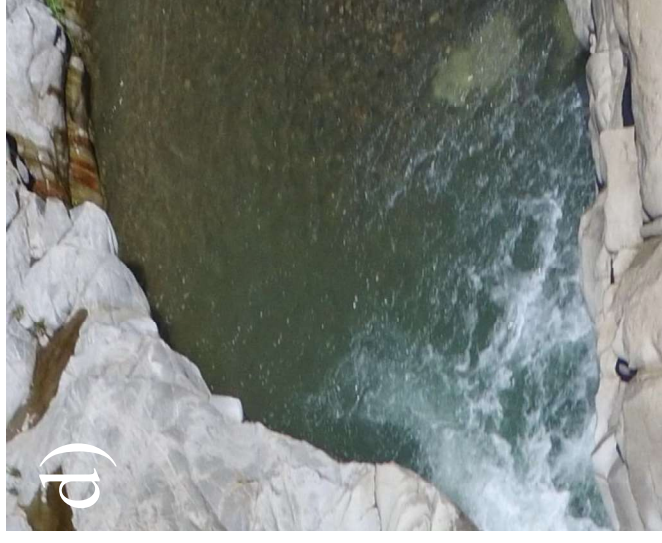
947 **Figure 12.** Correlations between geomorphic units index or values and ecological  
948 indexes for the fish community: a) mGUSI-D<sub>BC</sub> vs. Margalef diversity index vs.  
949 Shannon-Wiener and Simpson diversity indexes; b) Number of pools vs Shannon-  
950 Wiener and Simpson diversity indexes; c) number of steps vs Shannon-Wiener and  
951 Simpson diversity indexes. The correlation analysis was performed using the Pearson  
952 correlation at  $p < 0.05$ ).

953 **Figure 13.** Correlations between geomorphic units index or values and ecological  
954 indexes for the anuran community: abundance of *T. aerolatus* vs areal extension of  
955 riffles and pools (a and b); abundance of *O. mykiss* vs mGUSI-D<sub>C</sub> (c); and abundance of  
956 of *O. mykiss* vs number of cascades (d). The correlation analysis was performed using the  
957 Pearson correlation at  $p < 0.05$ .



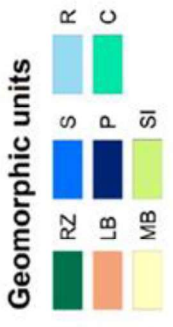
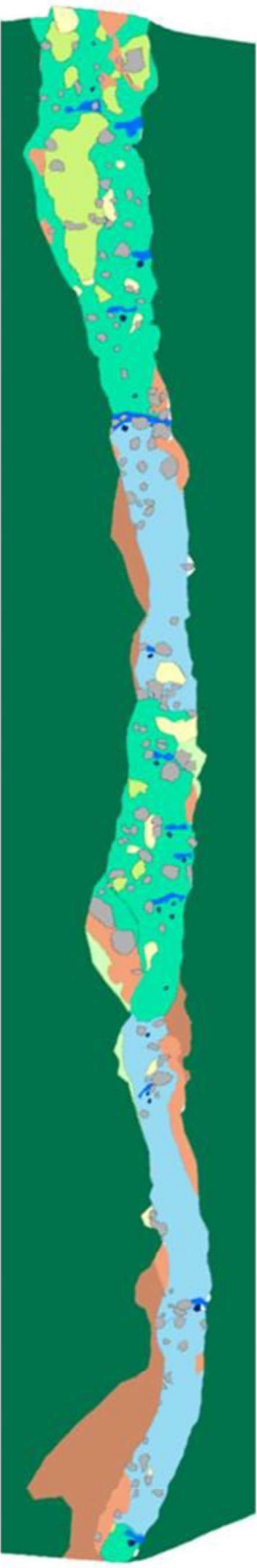
| Segment  | Reach  | Geomorphic Unit   | Microhabitat  | Spatial scales / Measured variables |  |
|--|--|---|---|-------------------------------------|--|
|  |  |   |   |                                     |  |
|  |  |  |  |                                     |  |
|  | Sediment grain-size<br>conductivity, dissolved<br>oxygen, pH, temperature            |   | Water depth, flow velocity,<br>sediment grain-size                                | Chemical-Physical<br>variables      |  |
| MQI<br>GUS (upscaled)  | GUS (survey)   | GUS (survey)  |   | Morphology                          |  |
| Analysis   | Analysis   | Analysis  | Sampling and analysis   | Macroinvertebrates                  |  |
| Analysis   | Survey and analysis  |   |   | Fish                                |  |
| Analysis   | Survey and analysis  |   |   | Amphibian                           |  |

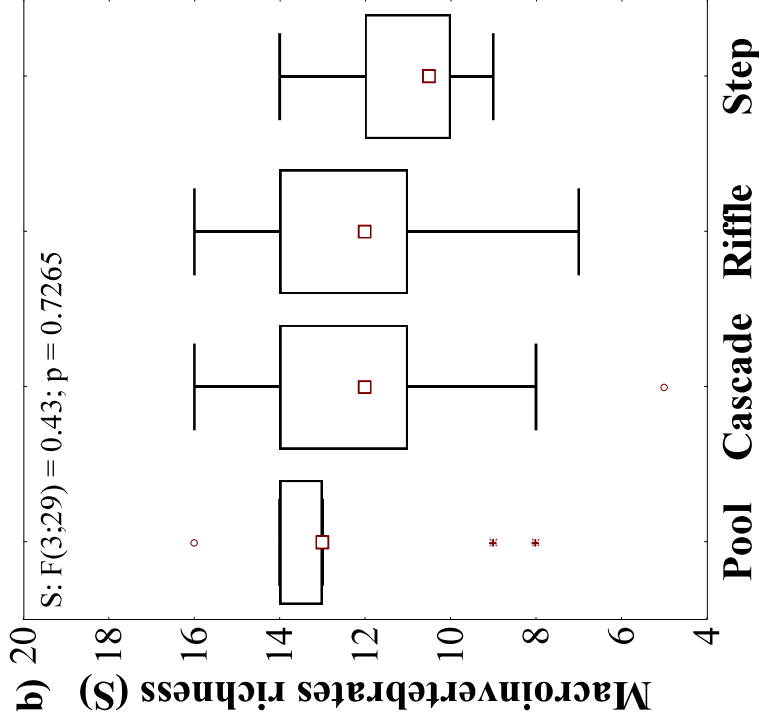
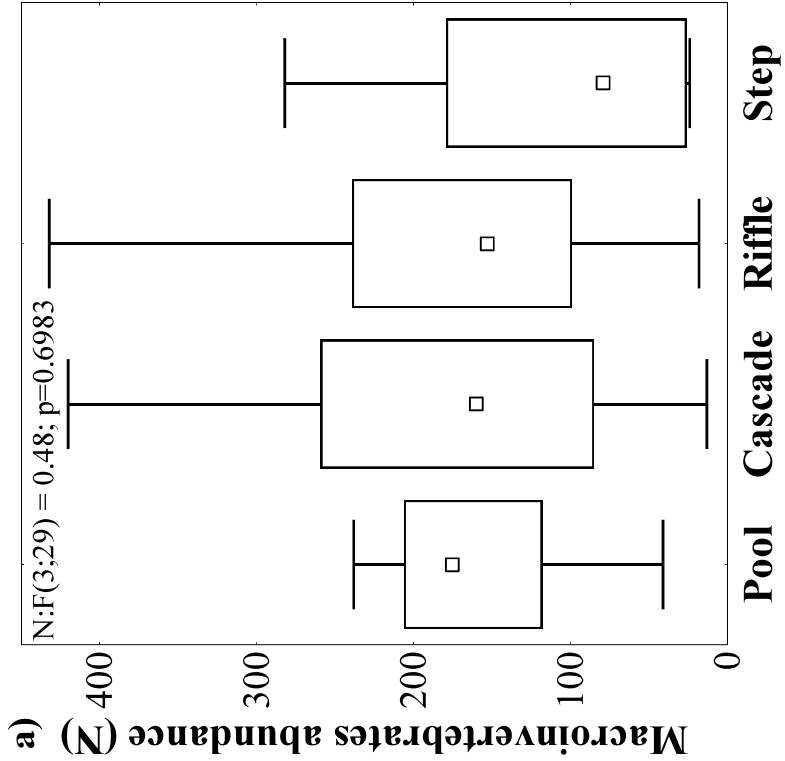












p = 0.0041

D:  $F(8;24) = 0.65$ ;  $p = 0.7238$

Chironomid:  $F(8;24) = 9.38$ ;  $p = 0.000009$

